Review Article

Aromaticrice andfactors affectingaromainrice

ABSTRACT

Aromatic rice is considered as an important value-added commodity with small variations in the aroma determiningpreference and unacceptability by the consumers. Aroma is the most desired trait followed by taste and elongation.Indiansub-continentishometoaromaticricediversity withnortheastbeingtherichest reservoir ofthegeneticvariability of aromatic rice. Aromatic rice has two groups, the long-grained Basmati type and the small and medium-grained indigenous aromatic varieties. 2-acetyl-1-pyrroline (2AP) is the principal component responsible for aroma,produced through a single recessive allele (*fgr*) at a locus on chromosome 8 which corresponds to the gene thatencodes betaine aldehyde dehydrogenase (*BADH2*). A premature stop codon upstream of key coding regions isintroduced by an 8-bp deletion in exon 7 which makes *BADH2* non-functional.The mutant *BADH2* transcript leadsto2APaccumulationinaromaticrice.The flowof**γ**-aminobutyraldehyde(GAB-ald)to**γ**-aminobutyricacid(GABA)is inhibited and consequently, the accumulated GAB-ald is diverted to a flavour component, 2-acetyl-1-pyrroline(2AP)byanon-enzymaticreactionwithmethylglyoxal.Accumulationof2-acetyl-1-pyrrolineinaromaticricedepends on the interaction of various genetic and environmental factors.Aromatic rice is prone to biotic and abioticstress. Grain quality of aromatic rice can be improved using existing aromatic genotypes by backcrossing, mutationbreeding or conventional cross-breeding. There is an urgent need to document, evaluate and conserve underutilizedgermplasmfor the improvementofaromaticriceinthefuture**.**

KEYWORDS:*2-acetyl-1-pyrroline,betainealdehydedehydrogenase,aroma.*

ABBREVIATION

2AP- 2-acetyl-1-pyrroline, *BADH2*-Betaine aldehyde dehydrogenase,GAB-ald-**γ**-aminobutyraldehyde,GABA-**γ**-aminobutyricacid, GC-FID-Gas chromatography - flame ionization detector,GC-MS- Gas chromatography–mass spectrometry, SPME-Solid phasemicro-extraction, TALEN-Transcription activator-like effector nucleases, CRISPR/Cas9- Clustered regularly interspaced shortpalindromic repeat-associated protein 9.

1. INTRODUCTION:

Aromatic riceis considered as animportantvalue-added commodity with small variations in the aromadetermining preference and unacceptability by the consumers. *Oryza sativa* has two major varietal groups, *indica*(*Hsien*) and *japonica* (*Keng*) that have been recognized in China since ancient times. Conventional scented ricevarieties consist of three isozyme groups viz., Group I (*indica*), Group V (*indica*), and Group VI (tropical *japonica*)[1]. Scented or aromatic rice is an important commodity worldwide and fetches premium pricesover non-scented varieties both in the local and international markets due to their superior grain quality and pleasantaroma.AromaisthemostdesiredtraitfollowedbytasteandelongationbyIndianconsumers.Indiansub-continentis home to aromatic rice diversity withnortheast being the richest reservoir of genetic variability of aromatic ricefrom the agro horticultural point of view. Aromatic rice bears special significance in the present globalized era due toits unique flavour and economic value.A diverse gene pool of aromatic rice is found in Assam which differs inthe intensity and durability of aroma, production potentialities and shape and size of grain. Aromatic rice landraces ofAssamareasuperiorclass underSaliorwinter riceknownasJoharice [2].

Aromatic rice has two groups, the long-grained basmati type and the small and medium-grainedindigenous aromatic varieties or landraces. Aromatic rice arising from diverse origins differs in the aroma. Areasunder cultivation of Basmati rice have stagnated as Basmati rice loses aroma and essential quality traits if grownbeyond stipulated tracts. In addition to the traditional long-grain basmati which is confined to North India, severalsmall and medium grain aromatic rice varieties are cultivated in India.There is a need to develop new aromaticrice varieties to maintain India's position in the global scented rice market to boost export. Only a few non-basmati scented rice varieties are traded in the international market while several are not, despite their eminentqualities such as elongation after cooking, aroma and agronomic characters [3]. Aromatic rice withvariations in the intensity of aroma, grain shape and size, durability, production potentialities etc. is found in Assam.Joha rice, an intermediate of typical Indica and Japonica class is amedium slender or medium broad category.Joha rice cultivars are known for its unique aroma, good cooking qualities, superfine kernel and excellentpalatability. Joha is popular due to its inherent scent and is high in demand for export. However, Joha rice is lessfavourable among the producers and the area under Joha rice is nominal owing to its low productivity and pooryielding ability. Joha rice is also prone to lodging, pest attack and takes relatively longer time to mature (120-160days).Joha grains are shorter (<4.7 mm) and have limited elongation (1.1 times) in comparison to other scentedrice in India. Joha grains have crude protein content in the range of 9.17-11.67 % in comparison to that ofBasmati.Variations in protein, amylase, amylopectin and mineral contents can be attributed to genetic factorsand environmental influences. Joha rice has ahigher content of albumin, globulin and amylase and is nutritionallycomparable to Basmati. High levels of albumin and globulin in aromatic rice play an important role in proteindigestibilityandalsoincreasethe biologicalvalue [4].

2. THEGENEFORTHE FRAGRANCEINRICE:

2-acetyl-1-pyrroline (2AP) is the principal component responsible for the fragrance, produced through asingle recessive allele (*fgr*) at a locus on chromosome 8 which corresponds to the gene that encodes betainealdehyde dehydrogenase (*BADH2*). A premature stop codon upstream of key coding regions is introduced by an 8-bp deletion in exon 7 which makes this gene non-functional (*badh2*).The mutant *badh2* transcript leads to 2APaccumulation in aromatic rice [3]. 2AP is a colourless to yellow solid and is categorized as flavor andthefragrancechemical compound [5]. The major compound responsible for the aroma inaromatic rice is 2-acetyl-1-pyrroline (2AP) which has a very low odour threshold making it possible for humans todetect it at minute concentrations. Oxidation of γ –aminobutyraldehyde (AB-ald), a 2AP precursor is catalyzed byBADH2 protein so that a nonfunctional allele results in the accumulation of both AB-ald and its cyclic form, delta-1pyrroline, which results in increase in synthesis of 2AP [6]. The 2AP level in the aerial parts ofplants is relatively higher in comparison to milled rice grains. The badh2 alleles in aromatic rice varieties all havecommoninsertions/deletionsandsinglenucleotidepolymorphismscomparedtonon-aromaticricevarieties,demonstrating acommon ancestorforallaromatic genotypes[7].

3. MAIN2APSYNTHESISPATHWAY:

Inthispathway,polyamines(arginine,ornithine,putrescineetc.)areconvertedtoGAB-ald(the immediate precursor of γ–aminobutyric acid, GABA) which spontaneously cyclises to delta-1-pyrroline. Delta-1- pyrroline is an immediate precursor of 2AP and a key factor for regulating the rate of biosynthesis of 2AP.In non-aromatic rice the functional BADH2 enzyme (coded by*OsBadh2*) converts GAB-ald to GABA which inhibitsthe biosynthesis of 2AP. In aromatic rice, due to non-functional badh2 enzyme (coded by *osbadh2*) GAB-ald cannot beconverted to GABA which results in the accumulation of GAB-ald leading to the formation of 2AP [8].

4. DETECTIONOFAROMA:

Detection of aroma compounds in rice is done both quantitatively and qualitatively. The fragrance ismeasured on a scale of one to ten using sensory panelists scalingthe fragrance or by utilization of a combination ofsensorypanelistsandgaschromatographicmethodstomeasurethelevelof2APinplantsamples.Tocategorizethe fragrance phenotype,a binary system of fragrant/non-fragrant is used which is a simple method.The reaction of seeds and leaf tissue to Potassium hydroxide is used as a common and convenient method toevaluate aroma. Concentration of 2-AP is determined by gas chromatography - flame ionization detector (GC-FID) or gas chromatography–mass spectrometry (GC-MS). Volatile components are analysed by solid phasemicro-extraction (SPME) fibers in combination with gas chromatography–mass spectrometry. SPME is usefulinscreening but not quantification of 2-acetyl-1-pyrrolinein aromatic ricevarieties [1].

5. FACTORSAFFECTING RICEAROMAANDFLAVOUR

5.1. GENETICS

2-acetyl-1-pyrroline (2AP) is believed to accumulate in rice due to an eight-base-pair (8-bp) deletion onchromosome 8 that encodes a putative betaine aldehyde dehydrogenase 2 (BAD2) leading to the fragrance in rice[9].464 varieties of rice were evaluated to quantify 2AP and the presence or absence of the fragrance allele(*fgr*). The 8-bp deletion was absent in several cultivars studied in spite of containing 2-AP suggesting that at least one other mutation influences the accumulation of 2AP other than the 8-bp deletion in fgr causing aroma in rice. Identification of multiple mutations for 2AP will enable selection for multiple genetic sources of 2AP which will help rice breeding programmes to develop highly aromatic varieties of rice [10].

5.2. PREHARVEST:

5.2.1. ENVIRONMENT:

Absence of favourable condition during aromatic rice growth stage in different environments leads todifference in aroma expression. Further studies need to be carried out to reveal the involvement of enzymesresponsible for the aroma and environmental mitigation to connect the genetic, molecular and chemical aspects ofaromaticrice [8].

5.2.2. RESPONSETOABIOTICSTRESS:

Aromaticriceishighlysensitivetoabioticstressmainlysalinity,drought,extremetemperature,submergencean dultravioletirradiation,whichreducecrop productivity andgrainquality [11].A number of protective mechanisms involving proline, methylglyoxal, GABA and calmodulin are present in rice plantswhich act as signaling molecules and protective agents during stress conditions. Polyamine degradation pathway(functional *OsBadh2* gene) or GABA-shunt pathway (activity of *OsGAD* genes) produces GABA. During stressconditions, GABA-shunt pathway becomes more active and GABA binds to the GABA-like receptor and releasesCa^{2+} from intracellular storage to increase Ca^{2+}accumulation in cytosol. As a result, the $Ca^{2+/}$CaM complex andstress response signal is amplified which induces stress response genes. Aromatic and non-aromatic rice withspecifically inhibited *OsBadh1* and *OsBadh2* genes are more susceptible to salt stress than wild-type with normalgene expression. 2AP content increases due to drought stress during grain formation and salt stress duringvegetativegrowth.Theincreasein2APcontentduringstressconditionsisduetoemissionofthestarch-boundand free form of 2AP. GABA can be produced only by the GABA-shunt pathway in aromatic rice as the polyaminesto GABA pathway remains inactive due to non-functional BADH enzyme. Therefore, elite aromatic rice varietiesare susceptible to biotic and abiotic stresses. Factors such as soil, relative humidity, temperature, moisture contentand pH during flowering to maturity stages highly affect aroma quality and survival of aromatic rice plantsbyregulating glycolysis,2APandGABA-shutpathway[8].

5.2.2.1. RESPONSETOTEMPERATURE

The quality of aroma is affected by high (> 30 ºC), low (< 20 ºC) and optimum temperatures (20 ºC to

30ºC) temperature, with higher 2AP production at optimum temperature, environmental or suitable growing conditions[12]. Higher 2AP production occurs at optimum temperature because enzymes involved in themain 2AP biosynthesis pathway remain more active with a low evaporation rate of 2AP. Additionally, methylglyoxalfromglycolysispathwaycontributestoproduce2APbyreactingnon-enzymaticallywithdelta-1-pyrrolinebysignaling for maintaining the physiological process. Polyamines such as glutamate, proline, ornithine, etc. areconverted to delta-1-pyrroline content under optimum temperature conditionswhich remains in equilibrium to theGAB-ald concentration. Moreover, proline produced from glutamate can be converted to GAB-ald which is furtherconverted to 2AP insteadof GABA inaromatic rice due to the non-functional *osbadh2*gene [8].

5.2.2.2. SOIL:

Aromainricedecreasesorisabsentwhensoilp His<4.70or≥5.10,withoptimumpHinthe rangeof4.70 - 5.00 being suitable for the aroma. Aroma is influenced by soil texture with the highest aroma observed in sandysoil and loamy sand and the lowest aroma in clayey soils. Aroma is more in sandy soil due to dehydration whichmakes it moderately saline and under drought stress. This results in a high level of proline synthesis for rice toadapt and survive under water stress which in turn increases the synthesis of 2AP, leading to an increase in aroma.Therefore, sandy loam or loamy sand which is slightly acidic or a moderate salinity or slightly salty is suitable forhigheraroma [13].

5.2.3. CULTURALPRACTICES:

Aromaand flavor ofaromaticriceareinfluencedbyculturalmethodsorcultivationpractices [8].

5.2.3.1. NUTRIENTAPPLICATION

The effect of time-specific nutrient application on 2-AP during the developmentalphases of aromatic rice cultivar Yungengyou 14 was studied. The accumulation pattern of proline, 2-AP and nitrogen and therelationships among them regarding 2-AP accumulation were studied at three nitrogen levels (0, 30 and 60kg /ha)at the booting stage. The highest 2AP content was observed in ear axes and flag leaves at 17 and 30 DAF underall the nitrogen levels studied. High 2-AP and proline contents were observed in different plant tissues during theearly grain filling stage on the application of nitrogen at the booting stage[14]. Therefore, the grain aroma contents ofaromatic rice can be improved by an additional dose of nitrogen at the booting stage. Aroma in Basmati rice isdetermined by complex interactions among the recessive gene (badh2), soil conditions, climate and geographicallocation. Reduction in the quality of aroma in Basmati and other scented rice varieties has been observed whichmightbeattributedtodisturbanceinrhizosphericmicrofloracausedbytheexcessiveuseofinorganicfertilizersandadoptionofmodernagriculturepractices.Beneficialmicroorganismscan beutilizedwhichmodifyplantmetabolomics to improve plant growth and health to enhance rice aroma [15]. The amyloseand protein contents of rice are influenced by cultural practices and the environment which affect the flavor andaroma of cooked rice. Rice samples of the same cultivar having low protein had more flavour than samples withhighprotein [16].

5.3. HARVEST:

The concentration of 2-AP decreased with an increase in planting density and time of harvest. In order toselect the optimum time of harvest, paddy yield and grain qualities have to be considered in addition to aromacontent. 2-AP content decreased with time and was significantly affected by the temperature with higher 2-APconcentrations obtained with the shortest storage time of 3 months and the lowest storage temperature of –4°C[17].

5.4. POST-HARVEST:

5.4.1. DRAINANDHARVESTDATES

Early drainage of fields may lead to moisture stress in grains before they attain physiological maturity.Moisture stress in grains will affect metabolic processes and in turn, volatile flavour compounds. Early harvest withhigher moisture contents and delay in drying leads to microbial growth and off-flavour metabolites. Aroma andflavourdecline with maturity [16]. Itani*et al.* (2004) [18] studied the effect of harvest time on theconcentration of 2-AP in two cultivars. It was observed that the concentration of 2-AP reached a peak at four or fiveweeksafterheading(WAH)inearly-headingcultivarandfourWAHinalate-headingcultivar.The2-APconcentration decreased rapidly to 20% and 40% of the maximum in early and late heading cultivar respectively.Champagne *et al.* (2005) [19] studied the effects of drain and harvest dates on sensory properties of rice and observedstable flavor withtimingof field draining(14 dayspan)andharvesting(32-48daysafterflowering).

4.4.2. HARVESTMOISTURECONTENT

Paddy may be held for more than 24 hours after harvest and before drying at moisture contents rangingfrom 16 to more than 26%.The growth of microbes may produce volatile compounds which affect the aroma or flavor ofrice[16].

4.4.3. DEGREEOFMILLING

The aroma of milled rice varies with the degree of milling [16]. Increased milling from 8 to14% led to a higher sweet taste and lower hay-like flavour, raw rice flavour, wet cardboard flavour and bitter [20]. The effect of the degree of milling on the flavour attribute intensities of three rice varieties werefound to be dependent on moisture content and variety or location. Intensities of desirable and undesirable flavor attributeswerehigherin ricedriedto 15%moisturecomparedto 12%[16].

4.4.4. MILLEDRICESTORAGETEMPERATUREANDTIME

Stale flavor isdevelopedinmilledriceduringstorageduetohydrolysisofsurfacelipidstoformfreefatty acids which are susceptible to oxidation. Free fatty acid formation also occurs due to the presence of lipaseof residual bran on the surface of milled rice. Moreover, oxidation of unsaturated fatty acids and the formation ofdifferent secondary oxidation products contribute to the development of odours and off flavours. The process isenhanced by milling by disruption of cells and release of lipoxygenase [9].Muthikul*et al*. (2019) [21] studied the effect of drying and storage times on aroma quality of Khao Dawk Mali-105 (KDML). It was observedthat drying time did not affect aroma characteristics of KDML fragrant brown rice (KBR) significantly. Longerstorage time decreased the intensity of desirable aroma of uncooked and cooked KBR significantly while theintensity of undesirable increased significantly. It was observed that drying treatment and longer storage timedecreasedthe2-APcontentofKBR.Highintensityofdesirable aroma andlowerintensityofundesirableodourwasobservedincookedKBRdried at35°Cfor2and6hoursthan non-dryingtreatment.

4.4.5. DRYINGCONDITIONSOFROUGHRICE,FINALMOISTURECONTENTANDSTORAGE:

Thearomaand flavor ofriceaftermillingisaffectedbythestoragetimeandtemperature.The concentration of 2-AP decreases if rice is dried at high temperature [16]. Wongpornchai*et al.*(2004) [22] studiedtheeffectofstoragetimeanddryingmethodsonaromaofaromaticricevariety,KhaoDawkMali-105. It was observed that the concentration of 2-acetyl-1-pyrroline decreased with an increase in storage time.Lowertemperature provided higher2-acetyl-1-pyrroline and lowerconcentrationof off-flavourcompounds.

6. DISAPPEARANCEOFAROMATICRICEVARIETIES:

The introduction of high-yielding and better-quality varieties has shifted the research focus from aromatic tomodern varieties of non-aromatic rice which has led to subsistence farming of aromatic varieties.The decreasein area under the cultivation of aromatic rice in addition to low productivity, pest susceptibilityand unavailability of qualityseeds as a result of increasing erosion of genetic diversity discourage farmers from cultivation of aromatic rice.Economicconstraintssuchaslimitedfinancialsupportfromfinancialinstitutions,lackofbetterinfrastructureand transportation facilities for processed rice are faced by the farmers. Erosion of aromatic rice diversity is a hugeproblem which is mainly due to small farm sizes as a result of which farmers are able to cultivate only a smallquantity of a particular aromatic rice variety. Inability of the farmers to retain the produce for seeds furthercontributes to the continuous disappearance of aromatic rice varieties. Due tothe non-replacement of seeds foryears together, seed quality gets deteriorated due to which several valuable aromatic rice varieties in the past 20yearshaveeitherbecomeextinctorareontheverge ofextinction[23].

7. CONCLUSION:

Aromaticriceisgenerallygrownforsubsistenceagricultureandleadstonon-existenceofwell-developed market. With the utilization of modern agricultural methods, several cultivars have been replaced bya lesser number of varieties.The intensityand type of aroma determine the uniqueness of aromatic genotypes which is dependent on genetic architectureand the agro-climatic factors. Protection of high-value aromatic genotypes requires active participation of farmers. India is home to several indigenous aromatic short graincultivarsand landraceswithmost of them being area specific with states having their own aromatic rice varieties.The short-grained aromatic rice varieties aremostly used by local consumers due to the lackof export potential. Several aromatic rice varieties have beenlostand are on the verge of extinction especially the small and medium-grained non-basmati aromatic rice. There isanurgentneedtodocument,evaluate,conserveandutilizethe underutilizedgermplasmforimprovementofaromaticriceinthefuture.Aromatic rice contains superior nutritional values and better amino acid profiles than non-aromaticvarieties.Fewshortgrainaromaticvarieties/landraceshavebetteraromaandotherqualitytraitsincomparisonto long grain types but are susceptible to various diseases with low yield due to the lack of a planned breedingprogrammefortheirgeneticmanipulation.Therefore,thereisaneedtoidentifyandpromotegeneticimprovement ofsuchshortgrainvarietiesforcultivationandmarketingglobally.Knowledgegainedbycharacterization and quantification of genetic diversity within and among closely related crop varieties will help inthe development of a suitable breeding program and management of plant genetics.Grain quality of aromatic rice can been enhanced by backcrossing, mutation breeding or conventional cross-breeding of existing aromatic genotypeswhich is time-consuming and laborious. Transfer of desirable genes to aromatic rice genotypes will assist toovercomethe limitationsofexistingaromaticricevarieties.Withconsumerpreferences playing a huge role, it is necessary to maintain quality checks from farm to table level. India plays ahuge role in the trade of aromatic rice globallyand therefore it isessential to understand the pattern of domestictradeofaromaticriceatthemicroleveltopromotetheircultivationandexport.Moreover,thereisaneedfor conservation and utilization of available germplasm for breeding new varieties while retaining the aroma quality.Keeping in view the demand for aromatic rice in the global market, aroma has beenconsideredbythebreedersfordevelopingcommerciallyimprovedricevarieties.Aromaplaysanimportantroleincon sumers' preferenceandmarketvalue.

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Author's contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.